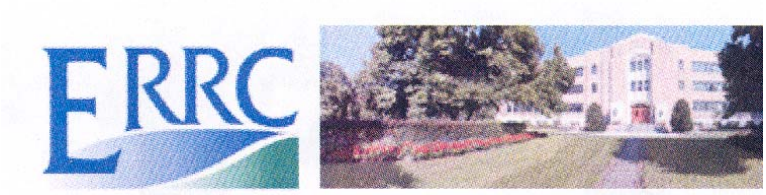

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Title: Suspending Lettuce Type Influences Recoverability and Radiation Sensitivity of Escherichia coli O157:H7

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Suspending Lettuce Type Influences Recoverability and Radiation Sensitivity of *Escherichia coli* O157:H7†

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ABSTRACT

An outbreak strain of *Escherichia coli* O157:H7 was inoculated onto closely related but structurally distinct types of lettuce (*Lactuca sativa*): Boston (butterhead lettuce), iceberg (crisphead lettuce), and green leaf and red leaf (colored variants of looseleaf lettuce). The *E. coli* O157:H7 was inoculated either onto the surface of cut leaf pieces or into a homogenized leaf suspension. Samples were gamma irradiated, and the radiation sensitivity of the inoculated bacteria was expressed as a *D*-value (the amount of ionizing radiation necessary to reduce the bacterial population by 90% [kGy]). The recovery of bacteria from nonirradiated leaf pieces was also measured. When inoculated onto the leaf surface, *E. coli* O157:H7 had significantly stronger radiation sensitivity on red leaf lettuce ($D = 0.119 \pm 0.004$ [standard error]) and green leaf lettuce ($D = 0.123 \pm 0.003$) than on iceberg lettuce ($D = 0.136 \pm 0.004$) or Boston lettuce ($D = 0.140 \pm 0.003$). When *E. coli* O157:H7 was inoculated into a homogenized leaf suspension, its sensitivity was significantly stronger on iceberg lettuce ($D = 0.092 \pm 0.002$) than on green leaf lettuce ($D = 0.326 \pm 0.012$), Boston lettuce ($D = 0.331 \pm 0.009$), or red leaf lettuce ($D = 0.339 \pm 0.010$), with a threefold difference. Significantly fewer bacteria were recovered from the surface of iceberg lettuce than from the surfaces of the other types of lettuce examined. Following radiation doses of up to 0.5 kGy, the texture (maximum shear strength) of lettuce leaves was measured along the midrib and along the leaf edge for each type of lettuce. There was no meaningful change in texture for any type of lettuce for either leaf section examined at any dose up to 0.5 kGy. These data show (i) that relatively subtle differences between lettuce types can significantly influence the radiation sensitivity of associated pathogenic bacteria and (ii) that doses of up to 0.5 kGy do not soften lettuce leaves.

Fresh produce has been associated with numerous outbreaks of foodborne illness in North America in recent years *(4)*. Salad vegetables, including fresh-cut lettuce, can be a source of pathogens such as *Escherichia coli* O157:H7, *Salmonella,* and *Shigella* spp. *(18),* and *E. coli* O157:H7 is known to grow on shredded lettuce stored at 12°C *(1)*. Ionizing radiation can eliminate pathogenic bacteria, including *E. coli* O157:H7 and *Listeria monocytogenes,* from vegetables *(13)*. The radiation sensitivity of bacteria can be influenced by the substrate upon which it is inoculated, such as different types of meats *(19),* various meat-based frankfurter formulations *(16),* or different species of sprouts *(14)*. Published studies suggest that even within a single commodity, such as lettuce *(7, 12),* potatoes *(2),* or blueberries *(9, 10),* the radiation sensitivity of associated bacteria or the product sensorial response may vary with the commodity variety or subtype.

The objectives of this study were to determine the influence of lettuce type on (i) the radiation sensitivity of *E. coli* O157:H7 on leaf surfaces and in a homogenized leaf extract, (ii) the attachment and recoverability of inoculated (nonirradiated) *E. coli* O157:H7 from leaf surfaces, and (iii) radiation-induced changes in the texture of leaf tissues.

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† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

MATERIALS AND METHODS

Pathogen. Reference cultures of the Jack-In-The-Box isolate of *E. coli* O157:H7 (ENT C9490; Centers for Disease Control and Prevention, Atlanta, Ga.) were maintained on 50% glycerol at −70°C. A frozen sample was regrown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) for 16 h at 37°C with agitation and streaked onto tryptic soy agar (TSA; Difco). This sample was incubated at 37°C for 48 h to form single colonies. These colonies were used to inoculate fresh tryptic soy broth for each experiment and were grown for 16 h at 37°C with agitation. The cell density of the starting inoculum was determined by serial dilution with sterile Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, Conn.) and pour plating with TSA. The cell density was typically 10^9 CFU/ml. The starting inoculum was used to inoculate leaf homogenate solutions directly. For the inoculation of leaf pieces, aliquots of 200 ml of starting inoculum were mixed with 1,800 ml of sterile BPB to produce the working inoculum.

Lettuce. Fresh produce was obtained from local markets on the day of each experiment. Four types of lettuce were used: Boston (butterhead), iceberg (crisphead), and green leaf and red leaf (colored variants of looseleaf or bunching lettuce) (Table 1). The outer leaves of each head were removed and discarded. Fully expanded mature leaves of each type of lettuce were weighed and then dried to determine the dry weight percentage. Fresh samples ($n = 5$) weighed 35 to 60 g. The material was processed at 60°C for at least 90 h in a drying oven. After processing, the samples of all lettuce types were of uniform dryness and brittleness. The experiment was performed twice.

TABLE 1. *Physical characteristics of Boston, green leaf, iceberg, and red leaf lettuces*

Parameter measured	Value for lettuce type			
	Boston	Green leaf	Iceberg	Red leaf
% dry wt	3.6 A[a]	5.9 B	3.4 A	3.7 A
Antioxidant power (μM FRAP/g)[b]				
Leaf fresh wt	45.7 A	72.8 B	54.5 C	83.6 D
Leaf dry wt	1,285.0 A	1,235.1 A	1,595.8 B	2,242.0 C
Leaf surface area/wt ratio (mg/cm^2)	25.6 A	29.2 B	35.9 C	19.9 D
Comments	Butterhead type, somewhat compact head, deeply involuted leaves	Looseleaf type, oblong leaves	Crisphead type, very compact head, relatively smooth leaves	Looseleaf type, red-colored variant, oblong leaves

[a] Measurements with different letters in the same row are significantly different ($P < 0.05$, analysis of variance).
[b] FRAP (ferric reducing/antioxidant power) equivalence: 1,000 μM ascorbic acid = 2,000 μM FRAP units.

To determine the effect of the varying topographical surface features of each lettuce type on the recoverability of inoculated bacteria, the surface area/weight ratio for leaves of each lettuce type was determined with digital imagery and an image analysis software package (SimgaScan Pro 5.0, SPSS Inc., Chicago, Ill.). Leaves of each type were rinsed thoroughly and dried in a salad spinner–type centrifuge (Oxo International, New York, N.Y.). The leaves were weighed and then torn so that each fragment would lie flat, thereby providing a two-dimensional picture of the three-dimensional leaf structure. The number of pixels occupied by each leaf fragment was measured, and the total number of pixels represented by the entire leaf was determined. On the basis of the known resolution of the image (150 dots per in., 22,500 dots per in^2), the number of pixels was converted into surface area (cm^2). This process was repeated for each of five leaves for each lettuce type, and the resulting data were used to determine a mean surface area/weight ratio (mg/cm^2) for each type of lettuce (Table 1).

Before the leaf material was used in the experiments, it was sanitized with a solution of 300 ppm sodium hypopchlorite at room temperature. The leaf material was submerged and gently agitated for 3 min. The leaves were thoroughly rinsed under running distilled water and spun in a sterile salad spinner–type centrifuge to remove excess surface water. The microflora of sanitized leaf material was measured for each lettuce type by a surface wash with BPB, serial dilution, pour plating with TSA, and incubation at 37°C for 24 h. The postsanitization population was found to be <20 CFU/g of leaf tissue.

Homogenized leaf tissue was used as a model solution to determine the effect of internal leaf chemistries on the radiation sensitivity of internalized bacteria. Homogenized leaf suspensions were prepared from fully expanded mature leaves that were sanitized as described. The leaf was detached from the head, and the basal portion of the leaf was removed approximately 5 cm from the attachment point. The leaves were roughly sectioned with a sterile knife, and 45 g of leaf material was placed into a sterile Oster-style blender jar (Thomas Scientific, Swedesboro, N.J.) with 180 ml of sterile BPB and blended at high speed using a laboratory-grade Osterizer-style blender (Thomas Scientific) for 5 s to completely homogenize the leaf material. The homogenate was poured through four layers of sterile cheesecloth into a fresh sterile beaker.

Noninoculated samples of the homogenized leaf tissue were assessed for their antioxidant power by the ferric reducing/antioxidant power (FRAP) assay *(3)*. Samples (50 μl) were placed in spectrophotometer cuvettes (five samples for each lettuce type), and 1.5 ml of fresh FRAP reagent solution was added; the solutions were fully mixed. The reaction was allowed to proceed for 6 min at room temperature to allow full development of the pigmentation. The absorbance of the reacted solution was read at 593 nm, and the value obtained was converted to μM FRAP equivalent using a previously determined standard curve (1,000 μM ascorbic acid = 2,000 μM FRAP) to account for dilution. The measurement of antioxidant power was expressed as μM FRAP/g of fresh leaf tissue and converted to μM FRAP/g of dry tissue on the basis of the previously determined fresh weight/dry weight ratio for each lettuce type.

Cut leaf pieces were prepared from the entire head after the outer leaves had been removed and discarded. The basal portion of the head was removed approximately 5 cm from the end. The leaves were sliced as a group into pieces weighing approximately 0.5 g. Cut pieces were sanitized, rinsed, and spun dry as described above.

Inoculation. The homogenized leaf suspension (99 ml) was inoculated with 1 ml of the starting *E. coli* O157:H7 culture. Aliquots (5 ml) of the inoculated suspension were dispensed into sterile glass tubes. A sterile buffer (BPB) was similarly inoculated for comparison. One tube per lettuce type per dose was used. The experiment was performed three times.

The cut leaf pieces of each type of lettuce were inoculated separately. Sanitized leaf pieces were transferred to a sterile glass inoculation dish (22 by 33 by 5 cm) in a biological airflow hood, and 1,000 ml of the working inoculum was added. The material was agitated gently for 120 s to completely submerge each piece and then transferred to a sterile salad spinner–type centrifuge (Oxo International). The material was spun twice to remove excess inoculum from the surface of the leaf pieces. Samples (45 g) of each type of lettuce were placed in no. 400 stomacher bags (Tekmar, Inc., Cincinnati, Ohio). The samples were refrigerated (4°C) until irradiation, typically for 30 to 60 min.

Irradiation. Temperature control was maintained during irradiation by the injection of gas-phase liquid nitrogen into the sample chamber. For the leaf homogenate, the samples were treated with 0.0 (control), 0.10, 0.25, 0.50, 0.75, and 1.0 kGy (one tube of homogenate per dose). Samples of iceberg leaf homoge-

nate were further evaluated with 0.0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5 kGy in separate, identical trials. For cut leaf pieces, the inoculated samples were treated with 0.0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5 kGy. In all cases, the irradiation was carried out at 4°C. Each study was performed three times. The samples were irradiated with a Lockheed-Georgia (Marietta, Ga.) cesium-137 self-contained gamma radiation source with a dose rate of 0.098 kGy/min. The dose rate was established with alanine transfer dosimeters from the National Institutes of Standards and Technology (Gaithersburg, Md.). Alanine pellets (Bruker, Inc., Billarica, Mass.) were used for dosimetry. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. The actual dose was typically within 5% of the nominal dose.

Sampling. After irradiation, the samples were refrigerated until microbiological sampling took place, typically for 60 to 90 min. Aliquots (1 ml) of irradiated leaf homogenates were serially diluted with sterile BPB. Pour plating with TSA was carried out to determine the extent of the surviving bacterial population. Three pour plates per dilution were incubated for 24 h at 37°C and counted with an automatic plate counter. For irradiated leaf pieces, sterile BPB (180 ml) was added to the stomacher bag and agitated for 60 s. A 1-ml sample was withdrawn for serial dilution with sterile BPB. The samples were diluted, pour plated with TSA, and incubated as described.

The data for each lettuce type were normalized against the control and plotted as the $\log_{10}$ reduction for the nominal doses. The slopes of the individual survivor curves were calculated by linear regression with a computer graphics program (SigmaPlot 5.0, SPSS). The ionizing radiation *D*-value (the radiation dose necessary to inactivate 90% of the population) was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp., Ottawa, Ontario, Canada).

Recovery from nonirradiated samples. In a separate experiment, nonirradiated samples of cut leaf pieces inoculated as described were similarly sampled after a surface wash with sterile BPB. These samples were diluted and plated with TSA and were taken to represent the recoverable bacterial counts. These counts were scaled by (i) CFU/g of leaf tissue or (ii) CFU/cm^2 of leaf tissue on the basis of the previously determined surface area/weight ratios for each lettuce type (Fig. 1).

Postirradiation texture. Whole, fully expanded, mature leaves were sanitized as described above. Identical circular 7.5-cm-diameter sections of the leaves were cut from (i) the leaf edge and (ii) the leaf midrib with a stainless steel cookie cutter. Five sections of each type were placed in no. 400 stomacher bags and given radiation doses of 0.0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5 kGy. The temperature during irradiation was 4°C. The samples were evaluated for maximum shear strength typically within 90 min of irradiation. Samples were analyzed with a TA-XT/2i5 texture analyzer using the TextureExpert 4.0 software package (Texture Technologies, Robbinsville, N.J.). The probe used was a Kraemer shear minicell. The experiment was performed three times, with independently prepared samples being irradiated concurrently.

Statistical analysis. The antioxidant power data (Table 1), surface area/weight ratio data (Table 1), *E. coli* O157:H7 recoverability data (Fig. 1), and texture data (Fig. 5) were evaluated by analysis of variance (SigmaStat, version 4.0, SPSS) using data pooled from the replications. For the *D*-value for each substrate type (Fig. 4), the significance of differences between the slopes for the regression lines was determined by analysis of covariance (Excel, Microsoft Corp., Redmond, Wash.) using data pooled from the replications.

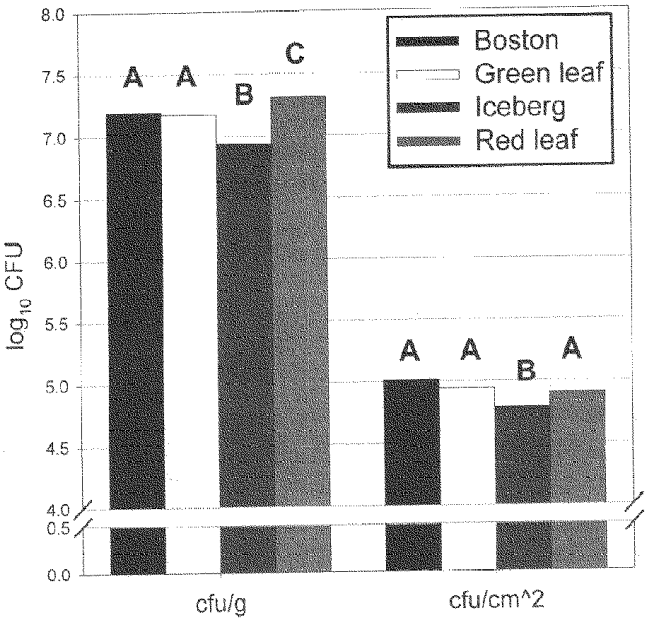

FIGURE 1. *Recovery of* E. coli *O157:H7 from the surfaces of four types of lettuce. Within each group, bars with different letters are significantly different* ($P < 0.05$, *analysis of variance).*

RESULTS

The four lettuce types differed significantly with regard to physical characteristics. Green leaf lettuce had a significantly higher percentage of dry matter than did the other types, whose percentages of dry matter did not differ from each other (Table 1). Every lettuce type was significantly different from every other lettuce type in terms of surface area/weight ratio, for which values ranged from 19.9 mg/cm^2 (red leaf) to 35.9 mg/cm^2 (iceberg) (Table 1). The antioxidant strength of each lettuce homogenate was significantly different from that of every other homogenate when expressed as μM FRAP/g of fresh tissue (Table 1). When scaled using the percentage of dry matter in the sample, the antioxidant strengths of Boston and green leaf lettuce were no longer significantly different from each other (Table 1).

In the absence of irradiation, significantly different numbers of *E. coli* O157:H7 CFU were recovered from the inoculated leaf samples of the four lettuce types (Fig. 1). When expressed as CFU/g of leaf tissue, *E. coli* O157:H7 populations on Boston and green leaf lettuce were not different, while iceberg lettuce had significantly fewer *E. coli* O157:H7 CFU/g and red leaf lettuce had significantly more CFU/g of leaf tissue. When *E. coli* O157:H7 counts were expressed as CFU/cm^2 of leaf tissue, the counts for Boston, green leaf, and red leaf lettuce were not different from each other, but iceberg lettuce had significantly fewer CFU/cm^2 than did the other types of lettuce (Fig. 1).

Linear regression adequately described the reduction of *E. coli* O157:H7 populations in lettuce homogenates (Fig. 2) and on leaf surfaces (Fig. 3). For all of the data sets, the R^2 value for the regression lines was at least 0.94. The radiation sensitivity of *E. coli* O157:H7 was significantly in-

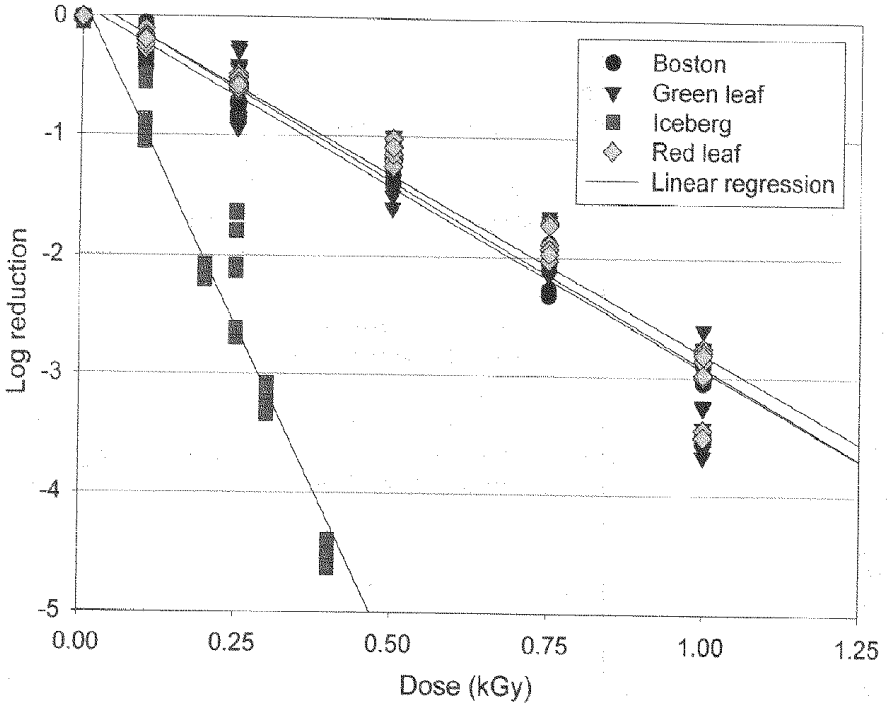

FIGURE 2. *Radiation sensitivity of* E. coli *O157:H7 suspended in homogenates of four types of lettuce.*

fluenced by the suspending medium. For Boston, green leaf, and red leaf lettuce homogenates, the *D*-value was significantly greater than that obtained in the phosphate buffer standard or in the iceberg lettuce homogenate (Fig. 4). The *D*-value obtained for leaf surface–associated *E. coli* O157:H7 was significantly greater for Boston and iceberg lettuces than for green leaf or red leaf lettuce (Fig. 4). The *D*-values for green leaf and red leaf lettuces did not differ significantly from that for the phosphate buffer standard.

The maximum shear force of leaf material was greater for samples taken from the leaf midrib area than for those taken from the leaf edge (Fig. 5). Radiation doses of up to 0.5 kGy did not induce a significant change in texture for midrib samples of any lettuce type or for leaf edge samples of Boston, iceberg, or red leaf lettuce (Fig. 5). Leaf edge samples of green leaf lettuce treated with 0.2 kGy had a significantly higher maximum shear force than did the control (0.0 kGy) leaf edge samples or the leaf edge samples treated with 0.4 kGy. There were no significant differences in shear force among the other dose levels.

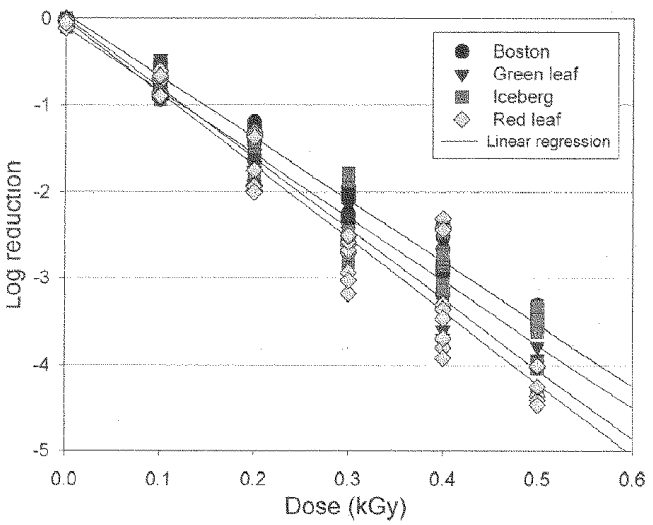

FIGURE 3. *Radiation sensitivity of* E. coli *O157:H7 on the surfaces of four types of lettuce.*

DISCUSSION

The radiation sensitivity of inoculated *E. coli* O157:H7 was significantly influenced by the type of lettuce with which it was associated and by the method of testing. The influence of the suspending medium on the radiation sensitivity of inoculated bacteria has been investigated for a variety of foods, including meat *(19)* and citrus juices *(11)*. Side-by-side comparisons of the sensory and microbiological responses to irradiation for different types or cultivars of a particular commodity are relatively lacking. In separate studies, iceberg lettuce *(7)* and romaine lettuce (an oblong

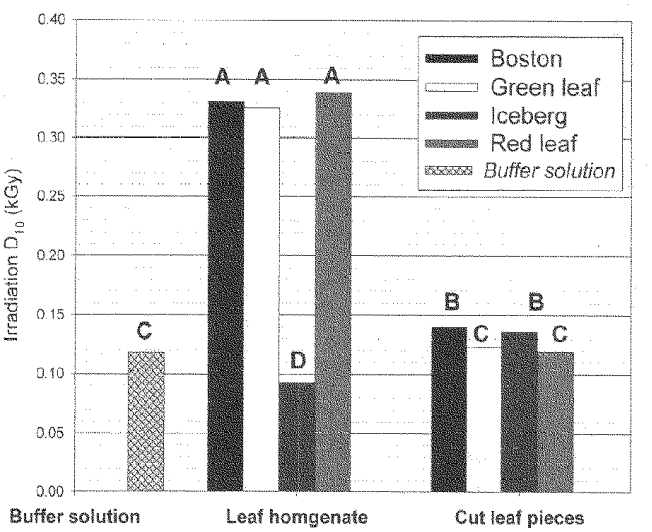

FIGURE 4. *Radiation* D*-values for* E. coli *O157:H7 in phosphate buffer, in lettuce leaf homogenates, and on leaf surfaces. Bars with different letters are significantly different* ($P < 0.05$, *analysis of covariance).*

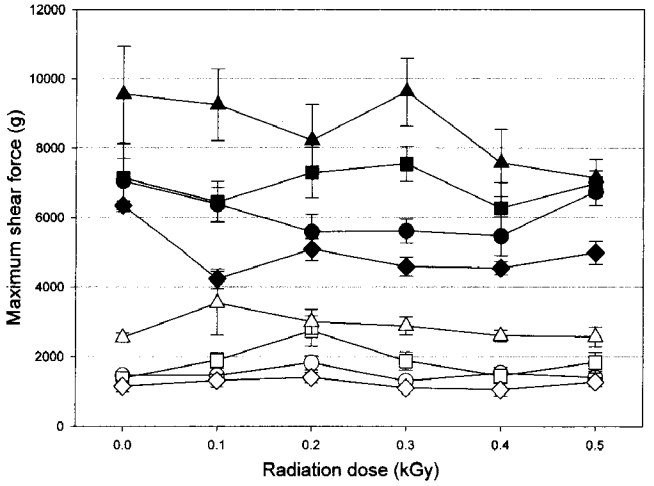

FIGURE 5. *Maximum shear force of four types of lettuce after irradiation: Boston (●), green leaf (■), iceberg (▲), and red leaf (◆). Measurements were taken from the leaf midrib (black) or the leaf edge (white). Bars indicate standard error.*

bib or cos type) *(12)* showed differences in respiration, textural response, and total aerobic plate counts following low-dose irradiation. A comparison of the potato varieties Ajax and Diamant *(2)* showed differences in the viscosity of starches after treatment with 0.20 kGy. In a series of studies involving blueberries, the flavor and texture of Sharpblue blueberries was considered acceptable after electron beam irradiation (1.0 kGy) *(9)*, while the flavor and texture of Climax blueberries similarly irradiated (1.0 kGy) *(10)* declined significantly. In a later study involving gamma irradiation, softening and quality loss after irradiation at 1.0 kGy were the same in the blueberry cultivars Brightwell and Tifblue; no negative impact was seen after irradiation at 0.5 kGy *(8)*. These studies, along with the data presented here, suggest that sensory and microbiological responses to irradiation are more strongly influenced by type or cultivar than previously recognized.

The *D*-value obtained for *E. coli* O157:H7 was significantly higher on the compact-head types of lettuce (Boston and iceberg) than on the looseleaf types (green leaf and red leaf). The mechanism by which this difference occurs has not been determined. Antioxidants are known to influence the antimicrobial efficacy of ionizing radiation by preferentially reacting with the radicals produced during irradiation, thereby protecting the bacteria *(15)*. However, the antioxidant powers of all of the lettuce types were determined to be relatively weak, and on a fresh-weight basis, the looseleaf types had more antioxidant power than did the compact-head types. Antioxidants in solution would tend to increase the *D*-value, suggesting that radical-scavenging compounds do not play a significant role in the mechanism governing the results obtained in this system. The *D*-values obtained for bacteria suspended in simple solutions are generally lower than those obtained on surfaces or in meats *(15)*.

The chemically complex leaf homogenates are a model for the type of chemistries that bacteria may be surrounded with following internalization. Internalized bacteria inhabit the intracellular spaces between cells of leaf tissue *(17)*. Given that the homogenate combines chemistries from the distinct anatomical regions of the leaf (intracellular fluid, cytoplasm, vacuoles, etc.), it is not a completely accurate model; however, it does provide a basic indication of the type of influence that internal leaf chemistries may have on the action of ionizing radiation. In this study, *E. coli* O157:H7 in Boston, green leaf, and red leaf homogenates was much less sensitive to radiation than was the same isolate suspended in iceberg lettuce homogenate, with *D*-values of ~0.33 and 0.092 kGy, respectively. The underlying reason for this large difference in sensitivity has not been determined. As with surface-associated bacteria, the antioxidant power of the leaf homogenates would seem not to be a primary factor. Studies are in progress to investigate the chemical makeups of the various homogenates in order to elucidate the mechanism of this notable difference. Despite the acknowledged limited ability of these homogenates to fully mirror what may occur during the irradiation of cut lettuce leaf pieces, these data do suggest that the internal chemistries of different lettuce types may have a significant impact on the radiation sensitivity of internalized bacteria.

On the basis of the *D*-value obtained for the inoculated leaf surface, the amount of radiation necessary to achieve the 5-$\log_{10}$ reduction recommended by the Food and Drug Administration is ~0.7 kGy for Boston and iceberg lettuces, as opposed to ~0.6 kGy for green leaf and red leaf lettuces. A similar calculation based on *D*-values obtained from inoculated homogenates suggests a dose of ~1.7 kGy for Boston, green leaf, or red leaf lettuce, as opposed to ~0.5 kGy for iceberg lettuce. The former dose exceeds the current U.S. regulatory limits for irradiation of vegetable products (i.e., 1.0 kGy), while the latter dose does not. Of particular interest is the difference in recommended doses based on the *D*-values obtained from surface- and homogenate-inoculated bacteria. This difference suggests that irradiation protocols that are designed to target produce-associated bacteria must give careful consideration to the physical location of the bacteria on the product.

The four types of lettuce examined differed significantly with regard to their percentages of dry weight and their surface area/weight ratios, as may be expected for different agronomic types. On the basis of the levels of bacteria recovered from inoculated leaf pieces, the extent to which inoculated bacteria associate themselves with the leaf surface appears to be influenced by lettuce type. To a certain extent, this influence can be addressed by normalizing the samples for the differences in surface area available for association. The issue of the importance of such factors as differences in product topology has been raised by Beuchat et al. *(5)* in considering the various ways in which data may be presented (e.g., CFU/g versus CFU/cm^2). In this study, each of the four types of lettuce provided a distinct surface for bacterial association. Factors such as hydrophobicity, stomatal density, and trichome density and other physical, chemical, and anatomical factors may influence bacterial association. These factors may also be expected to influence the chemical attachment processes associated with biofilm formation *(6)*.

High doses of ionizing radiation can induce softening

in fruits and vegetables through radiolytic degradation of pectins *(20)*. However, Hagenmaier and Baker *(7)* demonstrated that doses of 0.2 and 0.5 kGy did not induce noticeable softening in iceberg lettuce. In this study, leaf samples taken from the midrib area showed no significant differences in maximum shear force for any type of lettuce at any dose examined. For Boston, iceberg, and red leaf lettuces, the samples taken from the leaf edges showed a similar lack of sensitivity to radiation, with no significant differences in shear force among any of the doses. For green leaf lettuce, the shear force of leaf tissue given one intermediate dose (0.2 kGy) was statistically greater than that of the nonirradiated controls or leaf tissue given 0.4 kGy; the samples given higher doses (up to 0.5 kGy) did not differ from the control. In general, radiation doses of up to 0.5 kGy had no impact on the texture of the lettuce types examined, and the maximum tolerable doses are therefore expected to be in excess of 0.5 kGy.

On the basis of this study's *E. coli* O157:H7 *D*-value data for leaf surfaces and textural data, an irradiation dose of 0.5 kGy would reduce bacterial populations by 3.6 to 3.8 $\log_{10}$ without significant loss of texture. Achieving the recommended 5-$\log_{10}$ reduction may therefore be possible with ionizing radiation alone; however, it is generally accepted that irradiation should be part of an overall antimicrobial strategy that eliminates pathogenic bacteria while preserving product quality. This study has shown that in determining how best to use irradiation as a tool to increase food safety, the specific nature of the product being irradiated must be considered on a product-by-product basis. The isolate of *E. coli* O157:H7 examined in this study showed significantly different radiation sensitivities for different suspending lettuce types. Moreover, the levels of recoverable bacteria varied among the lettuce types, suggesting differences in bacterial association. While additional research is required to fully elucidate the mechanisms that influence the radiation sensitivity of surface bacteria, it is hoped that these data will provide a basis for further research in this area.

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